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Seasonal Change in Cold Tolerance of the House Spider, *Achaearanea tepidariorum* (Araneae: Theridiidae)

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田中一裕[1)]: オオヒメグモの耐寒性の季節変化

Abstract Field-collected nymphs of *Achaearanea tepidariorum* displayed significant seasonal variation in the level of cold tolerance. The whole body supercooling point (SCP), the absolute limit of freeze avoidance, was ranged from −8.1°C in August to −20.1°C in January, and the median lower lethal temperature (LLT 50), and indicator of chilling tolerance, from −2.1°C in August to −13.7°C in January. Seasonal depression of LLT 50 began to occur one month earlier than that of the SCP, suggesting that these two changes are induced by separate processes. The cold tolerance strategy in term of the seasonal patterns of SCP and LLT 50 was discussed.

Introduction

Survival at subzero temperatures may be important for the spiders living in the temperate and arctic regions. At least two options to tolerate temperature below the freezing point have been exploited by the terrestrial arthropods. One is the freeze tolerance and the other is freeze intolerance or susceptible. The former can survive extracellular freezing, while the latter die when frozen (see recent reviews by BLOCK, 1990, 1991; LEE, 1991). To date, no freeze tolerant species have been found in spiders (see reviews by KIRCHNER, 1973, 1987; SCHAEFER, 1977), so that the freeze avoidance seems to be essential for their winter survival.

For the freeze intolerant species, the whole body supercooling point (SCP), a temperature at which the body water spontaneously freezes, has been considered to represent the absolute limit of low temperature tolerance. Earlier works on arthropod's cold hardiness were, therefore, forcused on the SCP determination. Recent investigations, however, show that freezing is not the only cause of the low temperature mortality. In several arthropod species, a high mortality occurs at temperatures much above the SCP (*e.g.* KIRCHNER, 1973; LEE & DENLINGER, 1985; KNIGHT & BALE, 1986; KNIGHT *et al.*, 1986; BALE *et al.*, 1988; ROJAS *et al.*, 1991; TANAKA & UDAGAWA, in press). Injury caused by low temperature in the freeze intolerant species thus consists of at least two components: freezing injury and chilling injury. For estimating the cold tolerance in a freeze intolerant species, therefore, not only the supercooling capacity, but also the chilling tolerance should be measured.

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Cold tolerance in spiders has been often measured in terms of SCP (*e.g.* KIRCHNER & KESTLER, 1969; KIRCHNER, 1973, 1987; HAGVAR, 1973; SCHAEFER, 1977; DUMAN, 1979; LEE & BAUST, 1985; BROMHALL, 1988; CATLEY, 1992), but rarely by the chilling tolerance (KIRCHNER & KESTLER, 1969; KIRCHNER, 1973; SCHAEFER, 1976). In this study, I examined the cold tolerance of the house spider, *Achaearanea tepidariorum*, by measuring both the supercooling capacity and the chilling tolerance, and characterized the cold tolerance strategy in terms of the seasonal variation of SCP and chilling tolerance.

Materials and Methods

The house spider, *Achaearanea tepidariorum*, is a species commonly occurs in Japan and also in various parts of the world (YAGINUMA, 1986). In northern Japan, both nymphs and adults pass the winter at exposed habitats such as out-side walls of buildings in a state of diapause (TANAKA, 1989, 1991, 1992). The overwintering sites are generally above the snow cover, so that we can roughly estimate the natural hibernaculum temperature from the air temperatures recorded by the local meteorological observatory.

Spiders were collected monthly on the campus of Hokkaido University, at Sapporo (43°03′N), Hokkaido, from August 1991 to June 1992. Although various sizes of nymphs and adults were found throughout the year in this locality (K. TANAKA, unpublished observations), only the middle-sized nymphs, weighing between 3 and 10 mg, were used for the present study.

The whole body supercooling point (SCP) and the median lower lethal temperature (LLT 50) were measured to estimate the seasonal change in cold hardiness of this species. For SCP determination, each spider was put into a geratine capsule attached with the tip of the thermocouple connecting to a recorder (Rikadenki, KB681H). The capsule was further covered by a plastic vial 4.5 cm in diameter and 8 cm in hight) for lowering the cooling rate to approx. 0.05°C/min. SCP was determined by a release of the latent heat due to ice formation within the spider's body. Six to 12 nymphs were used to determine the SCPs of each monthly collection.

For LLT 50 determination, spiders were exposed for 48 h to various temperature from −1°C to −18°C at 1°C intervals. After the treatment, they were gradually rewarmed to +18°C and were put into a glass tube (2 cm in diameter and 7 cm in hight) individually. Survival was assessed 1 week after exposure by their web building and feeding activity; only the spider building a normal web and showing normal predatory behaviour against to the introduced prey victims (*Drosophila melanogaster*) was considered as a survivor. LLT 50 was calculated from the mortality by probit analysis (TORII, 1954).

Results

Winter conditions

The monthly air temperatures recorded at Sapporo Meteorological Observatory indicate that the house spiders were frequently exposed to subzero temperatures in their natural habitats during the winter months (Table 1). The minimum air temperature during the study period was −12.3°C on 6 February and the minimum 48 h

mean temperature was −6.6°C on 24–25 January.

Supercooling point

The seasonal change in the whole body SCPs is shown in Fig. 1. The monthly

Table 1. Monthly air temperature at Sapporo (43°03′N) (Sapporo Meteological Observatory).

	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.
Average	21.5	18.4	12.7	4.7	−0.9	−2.4	−2.5	1.0	6.9	11.7	16.4
Maximum	30.1	28.7	23.2	15.6	12.2	5.5	7.4	14.3	18.1	24.4	28.3
Min. 48 h*	18.7	15.1	7.7	−1.6	−6.4	−6.6	−5.8	−3.3	2.6	6.2	13.7
Minimum	13.0	8.9	2.4	−4.7	−9.3	−11.3	−12.3	−8.0	−1.2	3.6	9.2

* Minimum 48 h mean temperature.

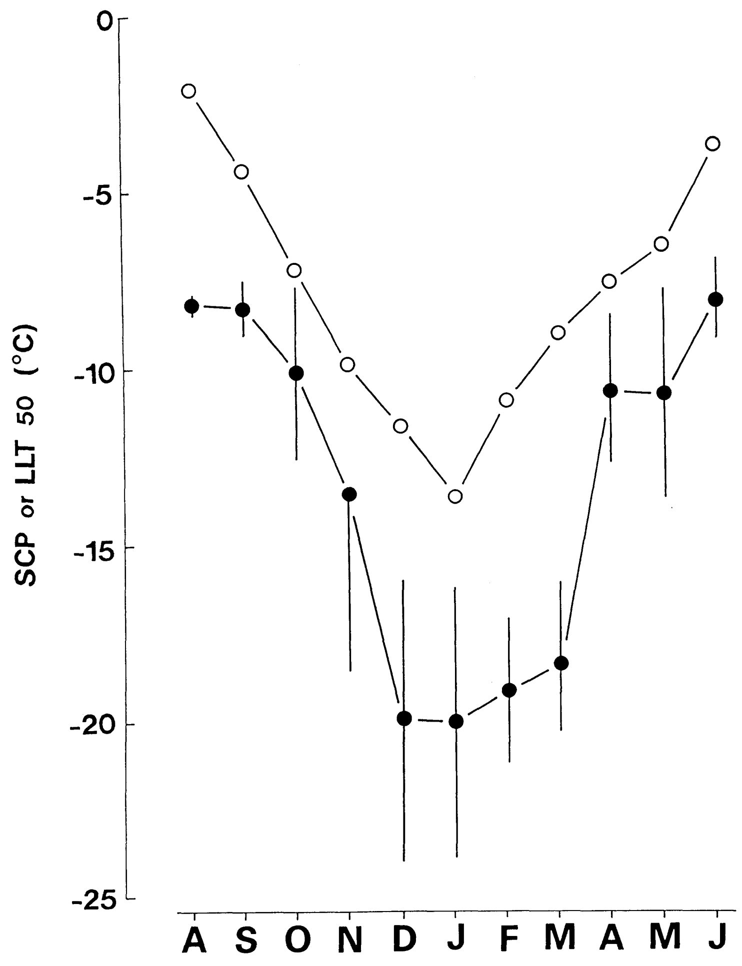

Fig. 1. Seasonal variation in the whole body supercooling point (closed circle: means± S.D.) and the median lower lethal temperature (open circle) of *A. tepidariorum*.

SCPs show significant seasonal variations in the mean values ranging from −8.1°C in August to −20.1°C in January (Kruskall-Wallis test, $P<0.01$). The SCP began to decrease in October and reached a minimum in January. It remained low until March and then remarkably increased in April.

To determine whether *A. tepidariorum* could tolerate tissue freezing or not, 12 individuals collected on January were cooled to −20°C at a rate of 0.05°C/min. During the cooling, 10 of 12 spiders froze and the remainder did not. Only unfrozen individuals recovered on return to +18°C, suggesting that this spider is freezing intolerant and the SCP represents the absolute limit of low temperature survival.

Although the unfrozen specimens resumed the walking activity after rewarming, they could not build a normal web and died within 5 days after cold exposure. This means that not only freezing, but also chilling is responsible for the low temperature mortality.

Lower lethal temperature

The seasonal change in the LLT 50 s is also shown in Fig. 1. Similar to the SCP, the LLT 50 showed apparent seasonal variation in the value ranging from −2.1°C in August to −13.7°C in January. The seasonal depression began to occur in September, one month earlier than the SCP, and reached to the minimum in January. In February and the after, it gradually returned to the summer level as the season progresses. Note that, throughout the entire experimental period, monthly LLT 50s were always higher than the corresponding monthly SCPs. This means that the significant portion of spiders died without freezing. This is well demonstrated in the dose mortality response curve of the January specimens to the 48 h cold exposure (Fig. 2). Despite of its lower monthly SCP (−20.1°C), a portion of spiders died at −13°C and none could tolerate the temperature below −16°C. Since no latent heat due to ice formation was observed in the dead specimens, this mortality seems to be caused by chilling injury.

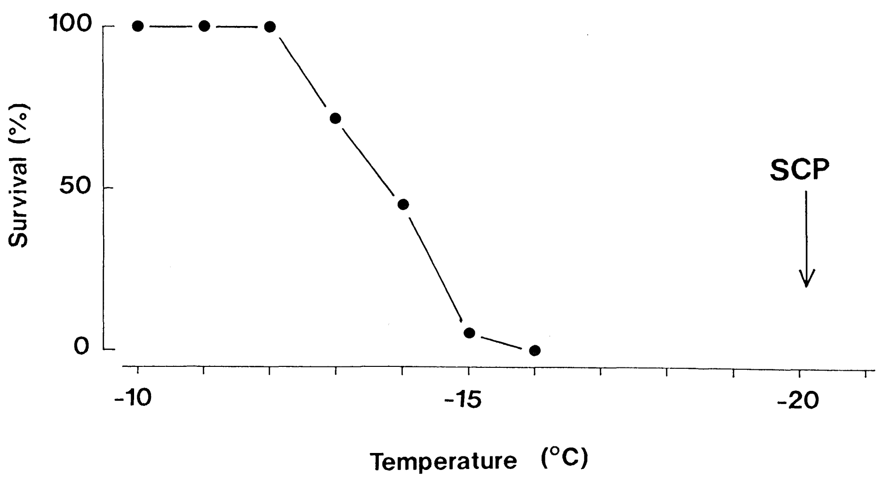

Fig. 2. Dose mortality response curve of *A. tepidariorum* collected on January to the 48 h cold exposure of various temperature regimes. Sample size was from 10 to 22.

Discussion

This study shows that *A. tepidariorum* is a freeze intolerant species, confirming the previous result reported by KIRCHNER (1973). The lowest monthly SCP of this spider was −20.1°C in January (Fig. 1). This value was significantly lower than the minimum air temperature during the study period (−12.3°C), indicating that the winter specimens have enough supercooling capacity to avoid lethal tissue freezing.

In the house spider, however, freezing was not the only cause of the low temperature mortality. From the present study, it was evident that this spider shows extensive chilling mortality. This means that prediction of winter mortality based solely on the supercooling capacity may be highly misleading as pointed out by KNIGHT *et al.* (1986).

Lower lethal temperature causing 50% mortality (LLT 50) is an adequate indicator of the chilling tolerance. In the grain aphid, *Sitobion avenae*, the laboratory estimated LLT 50 after 1–2 h cold exposure is well corresponding to the minimum grass temperature at which abrupt decrease of the field aphid density occur (KNIGHT *et al.*, 1986).

The lowest monthly LLT 50 of the house spider was −13.7°C in January. This value was nearly close to the minimum air temperature (−12.3°C), but was much lower than the minimum 48 h mean temperature (−6.6°C) (Table 1). Since the LLT 50 in this study was determined after 48 h cold exposure, not after a brief exposure represented as the daily minimum temperature, it might be appropriate to use the 48 h mean temperature as the corresponding hibernaculum temperature for predicting the chilling mortality. Even in the 48 h cold exposure, no chilling mortality occur at −12°C in the January specimens (Fig. 2). These observations suggest that the hibernating specimens have enough level of cold tolerance against not only freezing, but also chilling stress under the local climate.

The relationship between the SCP and LLT 50 is interesting. Under the field conditions, the seasonal depression of LLT 50 began to occur one month earlier than in the SCP (Fig. 1). Such differential performance of SCP and LLT 50 may imply that the changes in the supercooling capacity and the chilling tolerance are caused by separate processes. The independence of SCP from LLT 50 or other parameters of the chilling tolerance has been also reported in other arthropod species (LEE & DENLINGER, 1985; BENNETT & LEE, 1989; CHEN *et al.*, 1991; PULLIN *et al.*, 1991; TANAKA & UDAGAWA, in press).

Seasonal patterns of SCP and LLT 50 characterize the cold tolerance strategies. In the house spider, both the SCP and LLT 50 were enhanced in the approach to winter. In addition to the house spider, seasonal profiles of supercooling capacity and chilling tolerance have been reported in several freeze intolerant arthropods (KIRCHNER & KESTLER, 1969; WOUDE & VERHOEF, 1986; WOUDE, 1987; CHEN *et al.*, 1991; PULLIN *et al.*, 1991; TANAKA & UDAGAWA, in press). By the seasonal trends, those can be divided into two categories. One is the species enhances only the chilling tolerance in winter. The examples are the flesh fly, *Sarcophaga bullata* (CHEN *et al.*, 1991), the cabbage white butterfly, *Pieris brassicae* (PULLIN *et al.*, 1991) and the terrestrial isopod, *Porcellio scaber* (TANAKA & UDAGAWA, in press). The

other is the species, involving the house spider, enhances both the chilling tolerance and the supercooling capacity in winter. The temperate collembolans such as *Orchesella cincta* and *Tomocerus minor* (WOUDE & VERHOEF, 1986; WOUDE, 1987) fall in this category.

One of the factors determining whether a given freeze intolerant species enhances the chilling tolerance solely or together with the supercooling capacity may be the relative severity of the winter hibernaculum temperature to the summer SCP and LLT 50 (TANAKA & UDAGAWA, in press). This is well demonstrated in the terrestrial isopod, *Porcellio scaber*, from a more sheltered microhabitat in the same area. For the isopods, the winter hibernaculum temperature fall below the summer LLT 50, but remain higher than the summer SCP, due to the insulative properties of snow cover. Under these circumstances, there is no advantage to depress SCP in winter, so that the enhancement of the chilling tolerance solely would ensure the winter survival (TANAKA & UDAGAWA, in press).

This relationship may be also applicable to the house spider. The summer SCP (−8.1°C) and LLT 50 (−2.1°C) were significantly higher than the corresponding winter hibernaculum temperature (−12.3°C for SCP and −6.6°C for LLT 50). If the spiders maintain the summer SCP and LLT 50 throughout the year, they could not tolerate the freezing and chilling stresses during winter months. This may be a reason why the spiders lower both SCP and LLT 50 in the approach to winter.

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摘　要

オオヒメグモの耐寒性を，体組織の凍結開始温度（過冷却点）と冷温致死温度の面から解析した．本種には耐凍性はなく，体組織の凍結は致命的であった．しかし凍結にいたらなくても，一定時間冷温にさらされることでも死亡率は上昇した．過冷却点は夏の −8.1°C から冬の −20.1°C まで，また 50% 冷温致死温度は −2.1°C から −13.7°C まで変化した．いずれも1月に極値を示し，この時期に耐寒性が最も高まっていることが明らかになった．過冷却点と冷温致死温度の季節変化を，越冬場所の温度条件と関連づけて考察した．

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